CLAIM AMENDMENTS

1. (Currently Amended) A method for the detection and enumeration of microbes comprising:

a. exciting at least one intrinsic microbial fluorophore having a specific excitation range of electromagnetic radiation wavelength above 200 nm; whereby said ~~microbe containing~~ intrinsic fluorophore ~~fluorophores is~~ in the microbes is excited to emit fluorescence; and ~~fluorescence, and;~~

b. detecting the ~~fluorescence signals~~ signal intensities associated with the minima and maxima of the ~~excited~~ microbial ~~fluorophores, and~~ fluorescence; and

c. detecting the background intensities at the minima and maxima of the microbial fluorescence in the absence of excitation; and

d. calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and

e. subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal ~~excitation energies~~ intensities and measured background intensities from the detected signals of the ~~excited~~ microbial fluorescence; thereby ~~whereby~~ determining the ~~enumeration~~ number of microbes ~~is determined~~ by the magnitude of the detected fluorescence from which background, reflectance, and scattering contributions have been subtracted.

2. (Currently Amended) The method as set forth in Claim 1, wherein the ~~relative~~ ratios of multiple fluorescence signal intensities, from which measured

background and calculated reflectance and scattering have been subtracted, ~~detected, background-corrected, signals~~ are determined; whereby the detection of the physiological state of the microbes depends upon the requirement that the ratios of the background, scattering, and reflectance-corrected fluorescence signals lie within specified physiological ranges and that the enumeration of the microbes is determined by the magnitude of said detected signals the ~~whose relative~~ ratios of which lie within said expected ranges.

3. (Currently Amended) The method ~~A method~~ as set forth in Claim 1, wherein said ~~microbe fluorephores~~ microbial fluorophores are selected from the group consisting of nucleic acid polymers, tryptophan-containing proteins, tyrosine-containing proteins, adenosine triphosphate, calcium ~~dipicinolate~~ dipicolinate, reduced pyridine nucleotides, flavins, porphyrin-containing proteins, and other components excited in the 610-670 nm region.

4. (Currently Amended) The method of ~~Claim 1~~ Claim 1, wherein the viable microbes to be detected include at least one of the following: bacteria, fungi, protozoa, and rickettsiae; and the intrinsic microbial fluorophores used to detect the microbes include at least one of the following: nucleic acid polymers, tyrosine-containing proteins, tryptophan-containing proteins, adenosine triphosphate, reduced pyridine nucleotides, flavins, porphyrin-containing proteins, and others excited in the 610-670 nm region.

5. (Currently Amended) The method of ~~Claim 1~~ Claim 1, wherein non-viable microbes to be detected include at least one of the following: bacteria, fungi, ~~protazoe~~ protozoa, and rickettsiae; and the intrinsic microbial fluorophores used

to detect the microbes include at least one of the following: nucleic acid polymers, tryptophan-containing proteins, tyrosine-containing proteins, reduced pyridine nucleotides, flavins, porphyrin-containing proteins, and others excited in the 610-670 nm region.

6. (Currently Amended) The method of ~~Claim 1~~ Claim 1, wherein the microbes to be detected are bacterial endospores, ~~endospores~~ and the intrinsic fluorophores used to detect the endospores include at least one of the following: nucleic acid polymers, tyrosine-containing proteins, tryptophan-containing proteins, calcium dipicolinic acid, and others excited in the 610-670 nm region.

7. (Currently Amended) The method of ~~claim 1~~ Claim 1, wherein the microbes to be detected include viruses, and the intrinsic fluorophores used to detect the viruses include at least one of the following: nucleic acid polymers, tyrosine-containing ~~proteins~~ proteins, and tryptophan-containing proteins.

8. (Currently Amended) A method for the detection and enumeration of microbes comprising:

a. exciting ~~excitation of~~ multiple intrinsic microbial fluorophores with ultraviolet electromagnetic radiation having excitation wavelengths between 200 and 300 nm, whereby intrinsic fluorophores in any microbes present ~~containing intrinsic fluorophores~~ are excited to emit fluorescence, some of which ~~whose fluorescence~~ is self-absorbed to excite other microbial fluorophores that in turn emit fluorescence; and

b. detecting the fluorescence ~~signals~~ signal intensities associated with the minima and maxima of the ~~excited~~ microbial ~~fluorophores~~ fluorescence; and

c. detecting the background intensities at the minima and maxima of the microbial fluorescence in the absence of excitation; and

d. calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and

e. subtracting ~~the fluorescence from the reflected/scattered~~ the calculated reflected and scattered signal ~~excitation energies~~ intensities and measured background signal intensities from the detected signals of the microbial fluorescence; and

f. determining that the ~~relative~~ ratios of the detected ~~background-corrected~~ fluorescence signals from which background, reflectance, and scattering contributions have been subtracted lie within physiological ranges, thereby ~~whereby the enumeration~~ determining the number of ~~the~~ microbes ~~is determined~~ by the magnitude of the detected fluorescence signals from which background, reflectance, and scattering contributions have been subtracted ~~whose relative~~ the ratios of which lie within physiological ranges.

9. (Currently Amended) The method ~~A method~~ as set forth in Claim 8, wherein the intrinsic microbial fluorophores of said microbes include one or more of the group consisting of nucleic acid polymers, tryptophan-containing proteins, adenosine ~~triphosphate~~ triphosphate, and calcium dipicolinate compounds.

10. (Currently Amended) The method ~~A method~~ as set forth in Claim 8, wherein secondary-excited microbial fluorophores include one or more of the group consisting of calcium ~~dipicinolate~~ dipicolinate, reduced pyridine nucleotides,

flavins, porphyrin-containing proteins, cellular components excited in the 610-670 nm region, and the like.

11. (Currently Amended) The method as set forth in ~~of Claim 8~~ Claim 8, wherein the viable microbes to be detected include at least one of the following: bacteria, fungi, ~~protazoa~~ protozoa, and ~~rickettsiae~~ rickettsiae, and the intrinsic microbial fluorophores used to detect the microbes are selected from the group consisting of nucleic acid polymers, tyrosine-containing proteins, tryptophan-containing proteins, adenosine triphosphate, reduced pyridine nucleotides, flavins, porphyrin-containing proteins, and cellular components excited in the 610-670 nm region.

12. (Currently Amended) The method of ~~Claim 8~~ Claim 8, wherein the non-viable microbes to be detected include at least one of the following: bacteria, fungi, ~~protazoa~~ protozoa, and rickettsiae; and the intrinsic microbial fluorophores used to detect the microbes are selected from the group consisting of nucleic acid polymers, tyrosine-containing proteins, tryptophan-containing proteins, reduced pyridine nucleotides, flavins, porphyrin-containing proteins, and cellular components excited in the 610-670 nm region.

13. (Currently Amended) The method of ~~Claim 8~~ Claim 8, wherein the microbes to be detected are bacterial spores and the intrinsic fluorophores used to detect the spores include at least one of the following: nucleic acid polymers, tyrosine-containing proteins, tryptophan-containing proteins, calcium dipicolinic acid, and spore components excited in the 610-670 nm region.

14. (Currently Amended) Apparatus for the detection of microbes in a sample on a non-living ~~surface~~ surface, ~~or~~ in ~~air~~ air, or liquid comprising:

a. means for directing electromagnetic radiation towards the sample, said means adapted to emit radiation at energies capable of exciting at least one intrinsic microbial fluorophore;

b. at least one detector for electromagnetic radiation capable of converting the emitted, or ~~reflected/scattered~~ reflected and scattered radiation into electrical signals, said detector adapted to detect electromagnetic radiation at wavelengths above 320 nm to detect the minima and maxima associated with the fluorescence emission of said microbial fluorophores; and

c. means for analyzing the electrical signals corresponding to the fluorescence of the intrinsic microbial fluorophores, and the ~~reflected/scattered~~ reflected and scattered excitation ~~energies~~ intensities to determine the presence of microbes.

15. (Currently Amended) The apparatus ~~method~~ as defined in ~~claim 1~~ Claim 14, wherein the electromagnetic waves are directed towards the microbes in time-modulated pulses.

16. (Currently Amended) The apparatus defined in ~~Claim 14~~ Claim 14, wherein the means for directing electromagnetic radiation includes means for time-modulating the electromagnetic radiation.

17. (Currently Amended) A method for the detection and enumeration of microbial proteinaceous toxins comprising:

a. exciting at least one intrinsic ~~microbial~~ fluorophore having a specific excitation range of electromagnetic radiation wavelength above 200 nm; whereby said fluorophores in the proteinaceous toxin ~~microbe~~ present ~~containing intrinsic fluorophores is~~ are excited to emit ~~fluorescence, and;~~ fluorescence; and

b. detecting the fluorescence ~~signals~~ signal intensities associated with the minima and maxima of the excited ~~microbial fluorophores, and;~~ fluorophores; and

c. detecting the background intensities at the minima and maxima of the fluorescence in the absence of excitation; and

d. calculating the intensities of the reflectance and scattering at the maxima of the fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and

e. subtracting the calculated ~~reflected/scattered~~ reflected and scattered ~~excitation~~ signal intensities ~~energies~~ and measured background intensities from the detected ~~signals~~ signal intensities of the ~~excited~~ ~~microbial~~ fluorescence; thereby ~~whereby enumeration of the microbial~~ determining the amount of proteinaceous toxin ~~is determined~~ by the magnitude of said detected ~~background-corrected~~ fluorescence from which background, reflectance, and scattering contributions have been subtracted.

18. (Currently Amended) The method ~~A method~~ as set forth in Claim 17, wherein said microbe ~~fluorephores~~ fluorophores are selected from the group consisting of tryptophan-containing proteins and tyrosine-containing proteins.

19. (Currently Amended) A method for the detection and enumeration of ~~non-viable bacteria and~~ spores and non-viable bacteria comprising:

a. exciting at least one intrinsic microbial fluorophore having a specific excitation range of electromagnetic radiation ~~having~~ wavelengths between 550 and 700 nm; whereby said ~~microbe~~ intrinsic fluorophores present in the spores

and non-viable bacteria ~~containing intrinsic fluorophores is~~ are excited to emit ~~fluorescence, and;~~ fluorescence; and

b. detecting the ~~fluorescence signals~~ signal intensities associated with the minima and maxima of the ~~excited microbial fluorophores, and;~~ fluorescence; and

c. detecting the background intensities at the minima and maxima of the ~~intrinsic~~ fluorophores in the absence of excitation; and

d. calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and

e. subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal intensities ~~excitation energies~~ and measured background intensities from the detected ~~signals~~ signal intensities of the excited microbial fluoresence; thereby ~~whereby~~ enumerating the ~~enumeration of non-viable bacteria and~~ spores and non-viable bacteria ~~is determined~~ by the magnitude of the detected fluorescence from which background, reflectance, and scattering contributions have been subtracted.

20. (Currently Amended) The method as set forth in Claim 19, wherein the ~~relative~~ ratios of multiple detected, background-corrected signals are determined; whereby the distinction between spores and non-viable bacteria depends upon the requirement that the ratios of the background-corrected fluorescence signals lie within specified physiological ranges and that the enumeration of spores is determined by the magnitude of said detected signals ~~whose relative~~ the ratios of which lie within said expected ranges.

21. (Currently Amended) The method ~~A method~~ of ~~Claim 19~~ Claim 19, wherein the intrinsic microbial fluorophores used to detect the non-viable bacteria and bacterial spores are selected from a group including flavins, porphyrin-containing proteins, and other components excited in the 610-670 nm region.

22. (Currently Amended) The method of ~~Claim 19~~ Claim 19, wherein the non-viable bacteria and bacterial spores are detected on surfaces, inside paper envelopes, through paper, in ~~solution~~ solution, and in aerosols.

23. (Currently Amended) A method for the detection and enumeration of spores and non-viable bacteria comprising:

a. excitation of multiple intrinsic microbial fluorophores with electromagnetic radiation having excitation wavelengths between 550 and 640 nm, whereby intrinsic fluorophores in any spores and non-viable bacteria present ~~containing intrinsic fluorophores~~ are excited to emit fluorescence, some of which ~~whose fluorescence~~ is self-absorbed to excite other spore fluorophores that in turn emit fluorescence;

b. detecting the fluorescence ~~signals~~ signal intensities associated with the minima and maxima of the ~~excited~~ microbial ~~fluorophores~~ fluorescence; and

c. detecting the background intensities at the minima and maxima of the intrinsic fluorophores in the absence of excitation; and

d. calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and

e. subtracting ~~the fluorescence from~~ the calculated ~~reflected/scattered~~ reflected and scattered ~~excitation~~ ~~energies~~ signal intensities and measured background signal intensities from the detected ~~signals~~ signal intensities of the excited microbial fluorescence; and

f. determining that the ~~relative~~ ratios of the detected, ~~background-corrected~~ fluorescence signals from which background, reflectance, and scattering contributions have been subtracted lie within physiological ranges, thereby ~~whereby~~ determining the number of ~~the enumeration of the non-viable bacteria and~~ bacterial spores and non-viable bacteria ~~is determined~~ by the magnitude of the detected fluorescence signals from which background, reflectance, and scattering contributions have been subtracted the ~~whose~~ ~~relative~~ ratios of which lie within physiological ranges.

24. (Currently Amended) The method ~~A method~~ as set forth in Claim 23, wherein the intrinsic microbial fluorophores of said microbes include one or more of the group consisting of flavins, porphyrin-containing proteins, and other components excited in the 610-670 nm region.

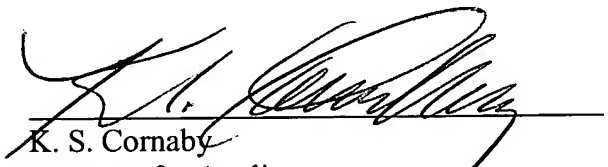
25. (Currently Amended) The method ~~A method~~ as set forth in Claim 23, wherein secondary-excited microbial fluorophores include one or more of the group consisting of intrinsic components excited in the 610-680 nm region, and the like.

26. (Currently Amended) The method of ~~Claim 23~~ Claim 23, wherein the non-viable bacteria and spores are detected on surfaces, inside paper envelopes, through paper, in ~~solution~~ solution, and in aerosols.

27. (Currently Amended) The method ~~A method~~ as set forth in Claim 23, wherein the spores and non-viable bacteria are detected inside paper envelopes and the secondary-excited microbial fluorophores are excited by emissions from the excited paper products.

Applicants respectfully request the Examiner's reconsideration of the rejection and passage of the claims to allowance. Should the Examiner have any questions, she is requested to call Applicants' undersigned attorney collect at (801) 521-3200.

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: LINDA S. POWERS and CHRISTOPHER R. LLOYD

SERIAL NO.: 10/054,419

FILED: January 2, 2002

FOR: METHOD AND APPARATUS FOR DETECTING THE PRESENCE OF MICROBES AND DETERMINING THEIR PHYSIOLOGICAL STATUS

GROUP ART UNIT: 1651

EXAMINER: SANDRA SAUCIER

AFFIDAVIT

STATE OF UTAH)
: ss.
COUNTY OF CACHE)

Comes now Christopher R. Lloyd, one of the inventors of the above-entitled invention for the application identified in Serial No. 10/054,419, and responds as follows:

Claim Rejections – 35 USC § 112 ("Section 112 Rejections")

Claims1-1 3, 15, 17-27 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 17, and 19 are indefinite because in step a, it recites "exciting at least one intrinsic microbial fluorophore *having a specific range of electromagnetic radiation wavelength above 200 nm.*" It is unclear if this refers to the excitation wavelength or the emission wavelength.

Response: The claims refer to excitation wavelength ranges. To definitely define the excitation of the sample, it is proposed that Claims 1(a), 17(a), and 19(a) read, "exciting at least one intrinsic microbial fluorophore having a specific excitation range of electromagnetic radiation wavelength . . ." It is believed that these changes to the Claims will provide a clearer and more definite description of the invention.

The independent Claims 1, 8, 17, 19, 23 use the term reflected/scattered excitation energies. It is unclear what the slash means between reflected and scattered. Are these terms interchangeable or are they distinct kinds of energies that are determined independently?

Response: The term 'reflected excitation energies' refers to the excitation photons that are reflected to the detector(s). The term 'scattered excitation energies' refers to the excitation photons that are absorbed and then emitted to the detector(s). These terms and their uses are known to those skilled in the art. (Scattered photons can either occur at the excitation wavelengths, or be of altered wavelength [inelastic

scattering].) Claims 1, 8, 17, 19, and 23 have been altered to read, "subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal ~~excitation~~ ~~energies~~ intensities. . ." Descriptions of the differences between reflection and scattering can be seen on pages 22-23 of The Basics of Spectroscopy, D. W. Ball (2001) SPIE, ISBN 0-8194-41040X, or in Chapter 16 of Spectrochemical Analysis, J. D. Ingle, Jr. and S. R. Crouch (1988) Prentice-Hall, Inc. ISBN 0-13-826876.

Claims 1 and 8 have passage language in step c or d respectively "whereby the enumeration of microbes *is determined* by etc.. Please use active language in method claims. For example, "determining the number of microbes etc.".

Response: To use the preferred active language, it is proposed that the Claims read, "subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal ~~excitation~~ ~~energies~~ intensities and measured background intensities from the detected signals of the ~~excited~~ microbial fluorescence; thereby ~~whereby~~ determining the ~~enumeration~~ number of microbes ~~is determined~~ by the magnitude of the detected fluorescence from which background, reflectance, and scattering contributions have been subtracted."

Claim 1 appears to be incomplete as the preamble only mentions detection of the microbes, not quantification (enumeration).

Response: It is proposed that the preambles of Claims 1 and 8 read, "A method for the detection and enumeration of microbes comprising:"

Step c in Claim 1 lacks clarity because it appears from the specification that the enumeration of the microbes is correlated to the detected fluorescence minus the

reflected/scattered energies and minus the background energies. However, the manner in which the claim is phrased does not properly reflect this critical element.

Response: To make Claim 1 reflect clearly and definitely the specification, it is proposed that the Claim 1 e [formerly 1(c)] read, "subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal ~~excitation~~ ~~energies~~ intensities and measured background intensities from the detected signals of the ~~excited~~ microbial fluorescence; thereby ~~whereby~~ determining the ~~enumeration~~ number of microbes ~~is determined~~ by the magnitude of the detected fluorescence from which background, reflectance, and scattering contributions have been subtracted." Similar changes have also been made in Claims 8, 17, 19, and 23.

Claim 2 lacks precedent for the recitation of "the relative ratios of multiple detected, background-corrected signals etc.". Thus, the reference to the independent claim is unclear.

Response: To clarify and more definitely reflect the specification (pp. 7, ¶ 2 and pp. 17), it is proposed that Claim 2 read, "The method as set forth in Claim 1, wherein the ~~relative~~ ratios of multiple fluorescence signal intensities, from which measured background and calculated reflectance and scattering have been subtracted, ~~detected, background-corrected, signals~~ are determined; whereby the detection of the physiological state of the microbes depends upon the requirement that the ratios of the background, scattering, and reflectance-corrected fluorescence signals lie within specified physiological ranges and that the enumeration of the microbes is determined by the magnitude of said detected signals the ~~whose~~ ~~relative~~ ratios of which lie within said expected ranges."

Claim 3 misspells "fluorophore" and "dipicolinate".

Response: The spellings of "fluorophore" and "dipicolinate" have been corrected in Claim 3.

Claims 5, 11, and 12 misspell "protozoa".

Response: The spelling of "protozoa" has been corrected in Claims 5, 11, and 12.

Claim 8 lacks clarity because in step d, it recites "whereby the enumeration . . . is determine by the magnitude of the detected signal", where it is the detected signal minus the reflected/scattered energies and background values, that is correlated with number of microbes.

Response: To make Claim 8 reflect clearly and definitely the specification, it is proposed that the Claim 8(f) [formerly 8(d)] read, "determining that the ~~relative~~ ratios of the detected ~~background-corrected~~ fluorescence signals from which background, reflectance, and scattering contributions have been subtracted lie within physiological ranges, thereby ~~whereby~~ ~~the enumeration~~ determining the number of ~~the~~ microbes ~~is~~ ~~determined~~ by the magnitude of the detected fluorescence signals from which background, reflectance, and scattering contributions have been subtracted ~~whose~~ ~~relative~~ the ratios of which lie within physiological ranges."

Claim 10 misspells "dipicolinate".

Response: The spelling of "dipicolinate" has been corrected in Claim 10.

Claim 17 is indefinite because in step c, the amount of toxin is determined by the magnitude of the fluorescence signals minus the background and the reflected/scattered energies, not merely the background-corrected fluorescence.

Response: To make Claim 17 reflect clearly and definitely the specification, it is proposed that the Claim 17 read, "A method for the detection and enumeration of microbial proteinaceous toxins comprising: (a) exciting at least one intrinsic ~~microbial~~ fluorophore having a specific excitation range of electromagnetic radiation wavelength above 200 nm; whereby said fluorophores in the proteinaceous toxin ~~microbe~~ present ~~containing intrinsic fluorophores is~~ are excited to emit ~~fluorescence, and;~~ fluorescence; and (b) detecting the fluorescence ~~signals~~ signal intensities associated with the minima and maxima of the excited ~~microbial fluorophores, and;~~ fluorophores; and (c) detecting the background intensities at the minima and maxima of the fluorescence in the absence of excitation; and (d) calculating the intensities of the reflectance and scattering at the maxima of the fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and (e) subtracting the calculated ~~reflected/scattered~~ reflected and scattered ~~excitation~~ signal intensities ~~energies~~ and measured background intensities from the detected ~~signals~~ signal intensities of the ~~excited~~ ~~microbial~~ fluorescence; thereby ~~whereby enumeration of the microbial~~ determining the amount of proteinaceous toxin ~~is determined~~ by the magnitude of said detected ~~background corrected~~ fluorescence from which background, reflectance, and scattering contributions have been subtracted."

Likewise, step c of Claim 19 is incomplete and indefinite.

Response: To make Claim 19 reflect clearly and definitely the specification, it is proposed that the Claim 19 read, "A method for the detection and enumeration of ~~non-viable bacteria and~~ spores and non-viable bacteria comprising: (a) exciting at least one intrinsic microbial fluorophore having a specific excitation range of electromagnetic

radiation ~~having~~ wavelengths between 550 and 700 nm; whereby said ~~microbe~~ intrinsic fluorophores present in the spores and non-viable bacteria ~~containing intrinsic fluorophores is~~ are excited to emit ~~fluorescence, and;~~ fluorescence; and (b) detecting the ~~fluorescence signals~~ signal intensities associated with the minima and maxima of the ~~excited microbial fluorophores, and;~~ fluorescence; and (c) detecting the background intensities at the minima and maxima of the ~~intrinsic~~ fluorophores in the absence of excitation; and (d) calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and (e) subtracting the calculated ~~reflected/scattered~~ reflected and scattered signal intensities ~~excitation energies~~ and measured background intensities from the detected ~~signals~~ signal intensities of the excited microbial fluoresence; thereby ~~whereby~~ enumerating the ~~enumeration of non-viable bacteria and~~ spores and non-viable bacteria ~~is determined~~ by the magnitude of the detected fluorescence from which background, reflectance, and scattering contributions have been subtracted."

Step d of Claim 23 also does not make clear that the detected signal minus the scattered/reflected and background energies is the value which is correlated with spore/non-viable bacteria mass/amount/numbers.

Response: To make Claim 23 reflect clearly and definitely the specification, it is proposed that the Claim 23 read, "A method for the detection and enumeration of spores and non-viable bacteria comprising: (a) excitation of multiple intrinsic microbial fluorophores with electromagnetic radiation having excitation wavelengths between 550 and 640 nm, whereby intrinsic fluorophores in any spores and non-viable bacteria present

~~containing intrinsic fluorophores~~ are excited to emit fluorescence, some of which ~~whose fluorescence~~ is self-absorbed to excite other spore fluorophores that in turn emit fluorescence; (b) detecting the fluorescence ~~signals~~ signal intensities associated with the minima and maxima of the ~~excited~~ microbial ~~fluorophores~~ fluorescence; and (c) detecting the background intensities at the minima and maxima of the intrinsic fluorophores in the absence of excitation; and (d) calculating the intensities of the reflectance and scattering at the maxima of the microbial fluorescence from the intensities of the background-subtracted minima with an appropriate algorithm; and (e) subtracting ~~the fluorescence from~~ the calculated ~~reflected/scattered~~ reflected and scattered ~~excitation energies~~ signal intensities and measured background signal intensities from the detected ~~signals~~ signal intensities of the excited microbial fluorescence; and (f) determining that the ~~relative~~ ratios of the detected, ~~background-corrected~~ fluorescence signals from which background, reflectance, and scattering contributions have been subtracted lie within physiological ranges, thereby ~~whereby~~ determining the number of ~~the enumeration of the non-viable bacteria and~~ bacterial spores and non-viable bacteria ~~is determined~~ by the magnitude of the detected fluorescence signals from which background, reflectance, and scattering contributions have been subtracted the ~~whose relative~~ ratios of which lie within physiological ranges."

Independent claims should begin with "A", while dependent claims should begin with "The".

Response: Claims 3, 9, 10, 18, 21, 24, 25, and 27 have been altered to reflect the proper use of "A" and "The".

Claim Rejections – 35 USC § 102 ("Section 102 Rejections")

The claims are directed to a method for the detection of microbes comprising:

a) **exciting at least one intrinsic microbial fluorophore with fluorescence emission wavelength above 200 nm to emit fluorescence,**

b) **detecting the fluorescence signals including the minima and maxima of the excited fluorophores,**

c) **subtracting the reflected and scattered excitation and background energies from the detected signals, whereby the enumeration of microbes is determined by the magnitude of the detected fluorescence.**

The references are relied upon as explained below.

U.S. 5,760,406 discloses a method of detecting microbes on a non-living surface comprising:

a) **exciting at least one intrinsic microbial fluorophore (NADH molecules) with a wavelength greater than 350nm,**

b) **detecting the fluorescence signals and the reflected or scattered energies (col. 2, 1. 1 4, col. 4, 1. 37), whereby the microbes are enumerated (Claim 7).**

U.S. 5,968,766 has essentially the same disclosure as '406.

Response: Claim 1 of U.S. Patent 5,760,406 states, "A method for the detection of microbes on a non-living surface comprising the steps of: (a) directing electromagnetic waves onto a non-living surface, said electromagnetic radiation having a wavelength greater than 350 nm, whereby any microbial cells present on the surface and containing nicotinamide adenine pyridine nucleotides are excited to emit fluorescence

having a higher wavelength and whereby some of said electromagnetic radiation is reflected by said surface, and (b) sensing the fluorescence and the reflected electromagnetic radiation as a measure of the amount of microbes present on said surface." Claim 7 of U.S. Patent 5,760,406 states, "A method is defined in Claim 1 which includes the step of sensing the fluorescence of microbial cells and sensing the electromagnetic radiation reflected by said surface and determining the difference or ratio therebetween as a measure of the amount of microbes present on said surface." Where the determination of the microbial content is found by calculating the ratio of the fluorescence to the reflectance (Claim 7 above) the method is different than that described in the disclosure. The following comments refer to detection that is based upon the difference between fluorescence and reflectance. U.S. Patent 5,760,406 utilizes the detection of reflectance-corrected NAD[P]H fluorescence to detect the presence of viable bacteria; in U.S. Patent 5,760,406 the contribution of reflectance is directly measured. The current application (1) requires the subtraction of background fluorescence and scattering effects in addition to any reflected excitation energies for the detection of numerous intrinsic fluorophores to find viable bacteria, nonviable bacteria, endospores, viruses, and proteinaceous materials, (2) measures the intensities associated with the minima of the intrinsic fluorescence, and (3) the subtracted reflectance and scattering is calculated (not measured) by means described in the application. In the current application the ratios of the detected and corrected fluorescence signals are used to verify the presence of microbes; in U.S. Patent 5,760,406 the ratio (or difference) of the fluorescence signal to the reflected signal is used for the detection of microbes. Additionally, U.S. Patent 5,760,406 discloses using the detected reflected

electromagnetic radiation to "normalize the [detected fluorescence] signal and compensate for variations in the signal . . ." The present application describes determining the magnitude of combined reflectance and scattering by measuring fluorescence at both the minima and maxima of the excited intrinsic fluorescences. The determination of the effects of reflectance, scattering, and background fluorescence for detection and enumeration of bacteria are done differently for the current application relative to U.S. Patents 5,760,406 and 5,968,766.

Claim Rejections – 35 USC § 103 ("Section 103 Rejections")

Claims 1, 3-6 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hill, et al.

The claims are directed to a method for the detection of microbes comprising:

a) **exciting at least one intrinsic microbial fluorophore with fluorescence emission wavelength above 200 nm to emit fluorescence,**

b) **detecting the fluorescence signals including the minima and maxima of the excited fluorophores,**

c) **subtracting the reflected and scattered excitation and background energies from the detected signals, whereby the enumeration of microbes is determined by the magnitude of the detected fluorescence.**

The references are relied upon as explained below.

Hill, et al. disclose a method of detecting microbes comprising exciting an intrinsic fluorophore (tryptophan) with a He-Cd laser @ 325nm with an emission

maxima @ 375nm (page 113). The magnitude of the detected fluorescence can be correlated to the number of microbes (biomass determination, page 108). The reflected/scattered excitation energy does not present a problem and is, in fact, zero (page 113). Therefore, it does not have to be subtracted from the fluorescence signal. It would be obvious to use this method whether the microbes are viable or nonviable because it is the tryptophan which fluoresces and is the basis for the quantitation of the microbes and the tryptophan would be present in the microbe whether the microbe were viable or non-viable.

One of ordinary skill in the art would have been motivated at the time of invention to make this measurement in order to obtain the results as suggested by the references with a reasonable expectation of success. The claimed subject matter fails to patentably distinguish over the state of the art as represented by the cited references. Therefore, the claims are properly rejected under 35 U.S.C. § 103.

Response: The report from Hill, et al. describes detection of microbes via detection of the intrinsic fluorescence from the amino acid tryptophan at 375 nm when excited with electromagnetic radiation at 325 nm. Hill, et al. teaches only the detection of one fluorescence signal, not detection of intensities at both minima and maxima of the microbial fluorescence as is described in the current application. Additionally, the **Initial Measurements** report of the **RESULTS** section of Hill, et al. (page 111) reports, "Because the fluorescence signal from tryptophane [sic] is relatively weak, the "bleed-through" of the excitation source was more intense than the fluorescent signal itself. Using narrower bandpass filters reduced the signal so much that a reasonable signal-to-noise ratio could not be maintained." Hill, et al. continued, "it was felt that this was not

worth pursuing further since there was no adjustment left for measuring weaker solutions." Results from these initial experiments were used as the justification for using LIF (laser induced fluorescence) to detect biomass. Hill and Angell may not have been so quick to abandon their initial experiments if they had been familiar with work done four decades previously (Chance & Thorell, *J. Biol. Chem.* (**1959**) 234(11): 3044-3050 and Duysens & Kronenberg, *Biochim. et Biophys. Acta* (**1957**) 26:437) in which the fluorescence signals from single cells were being obtained with microfluorometry using similar methods that they so quickly abandoned. Careful inspection of Hill, et al. shows that though they claim (page 113), "Nothing [being] observed" from the portion of the sample that did not contain biomass, that in fact reflected and scattered excitation energies were being exhibited. Using the data from Figure 4 (re-plotted below for clarity) shows from inspection that the intercept is <u>not</u> zero.

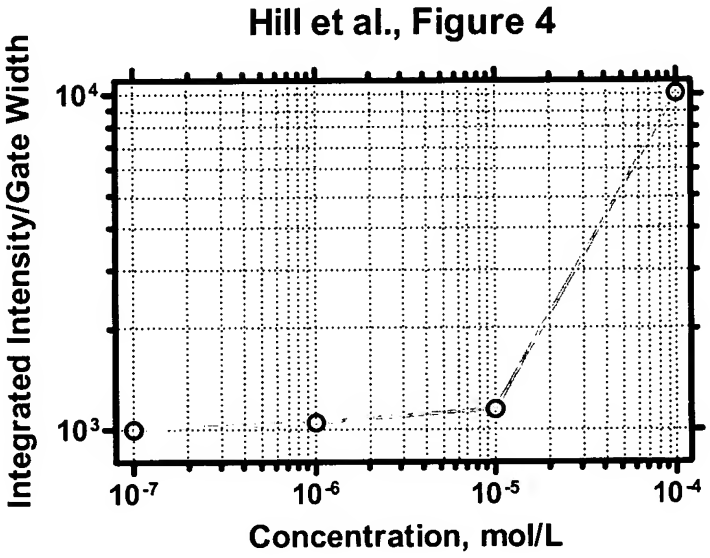
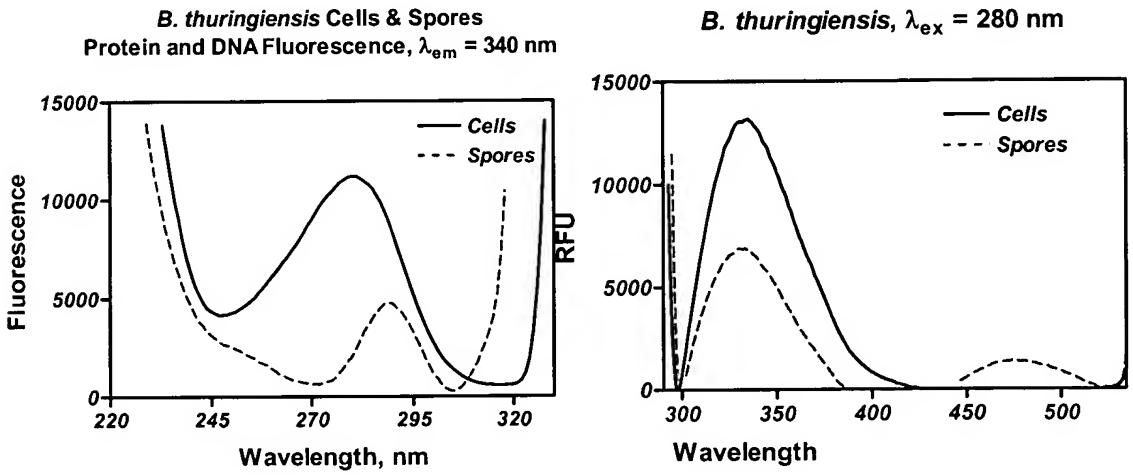


Figure 4 from Hill, et al. replotted.

Imaging of single cells (like that shown in Figure 5 of Hill, et al. or described in Chance, et al.) is known to those skilled in the art and can be achieved if the detection is done with so little sensitivity that reflection and scattering of the excitation photons (that do always exist) are not detected above the noise of the experiment. The results obtained by Hill, et al. are unsurprising as they used a laser line at 325 nm to excite tryptophan. The λ_{max} for tryptophan (from both the literature [pp. 14-15 in Proteins, 2nd Edition, T E. Creighton, c. 1993 W. H. Freeman & Company, ISBN 0-7167-2317-4] and experiment (see the Excitation and Emission Spectra of Microbes figures provided) is around 280 nm; the molar extinction coefficient of proteins in microbes is at least 20 times less at 325 nm. (The predicted signals from fluorescence and scattering are expected to be similarly reduced.) Thus, the contributions from scattering are expected to be extremely depressed when (1) fluorophores are not excited at their λ_{max}, and (2) fluorescence is not measured at the maximum energy (as is taught in the current application.) The methodology described by Hill, et al. is not applicable to detection of



Excitation and Emission Spectra of Microbes

microbes in solutions where scattering is expected to be relatively large or on surfaces exhibiting normally encountered background fluorescence.

Christopher R. Lloyd

Subscribed and sworn to before me this 17th day of September, 2003.

SHANNON WAGNER-BUTLER
Notary Public
State of Utah
My Commission Expires January 1, 2007
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My Commission Expires:

1/1/2007